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Supplemental Information

Abl Regulates Planar Polarized Junctional Dynamics through β-Catenin Tyrosine Phosphorylation

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Supplemental Inventory

Figure S1, related to Figure 1. Planar polarized localization of two additional phosphospecific antibodies in intercalating cells.

Figure S2, related to Figure 4. Quantitation of defective cohesion during actomyosin contraction in *abl* mutants.

Figure S3, related to Figure 5. Demonstration that E-cadherin planar polarity is disrupted in *abl* mutants.

Figure S4, related to Figures 6 and 7. Demonstration that the β-catenin C-terminal domain is not required for its localization or function during axis elongation. This construct was used in Figure 6 and Figure 7A-G.

Figure S5, related to Figure 7. Demonstration that *abl* knockdown and expression of unphosphorylatable (Y667F) or phosphomimetic (Y667E) β-catenin transgenes do not disrupt the striped pattern of engrailed expression.

Supplemental Experimental Procedures

Supplemental Information

Supplemental Data

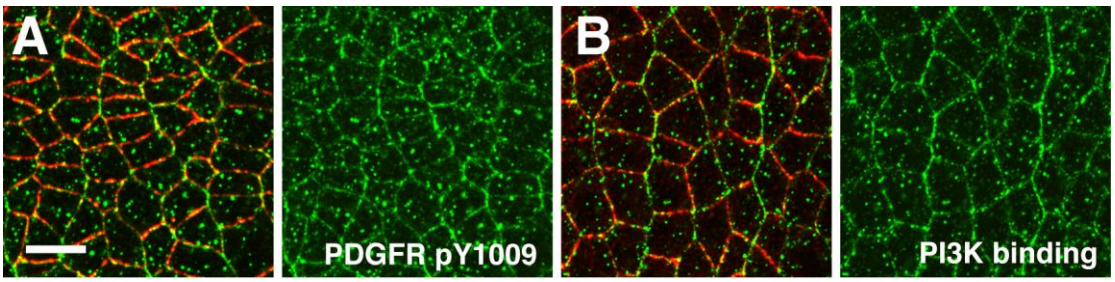

Figure S1, related to Figure 1. (A and B) Localization patterns detected by antibodies to human phospho-PDGF Receptor beta (Tyr1009) (green, A) and phospho(Tyr) p85 PI3K Binding Motif (green, B) in stage 7 wild-type *Drosophila* embryos (costained with Baz in red). Anterior left, dorsal up. Bar, 10 μm.

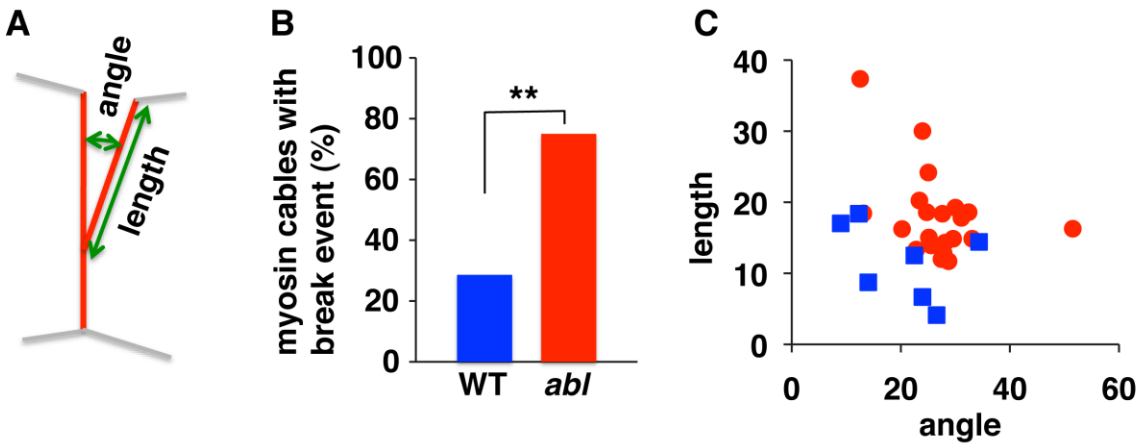

Figure S2, related to Figure 4. Defective cohesion during actomyosin contraction in *abl* mutants. (A) The angle and length of separation between cortical myosin signal in adjacent cells were measured in cases where apparent separations were detected. (B) The percentage of cables with apparent break events was greater in *abl* mutants (red, 21 breaks in 15 cables, n=20 cables analyzed) compared to wild type (WT) (blue, 7 breaks in 6 cables, n=21 cables analyzed) (p=0.0048). (C) For the experiment in B, the length and angle measurements are plotted for each apparent break event. Apparent breaks in WT (blue squares) are shorter in length on average than apparent breaks in *abl* mutants (red circles).

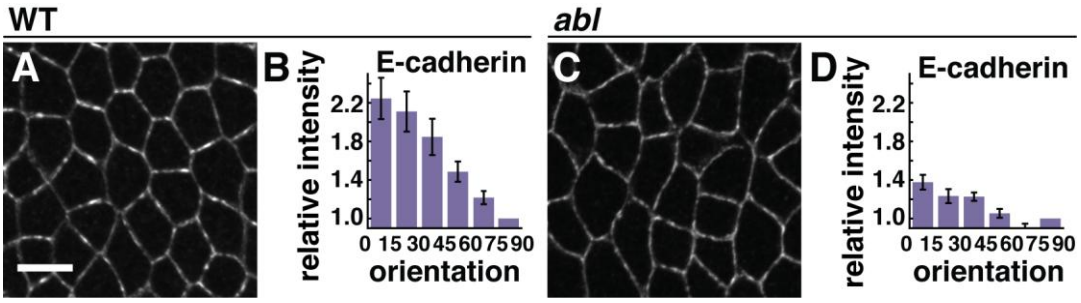

Figure S3, related to Figure 5. Abl is required for the planar polarized localization of E-cadherin. (A-D) E-cadherin localization in WT (A,B) and *abl* mutant embryos (C,D). Quantitation of E-cadherin planar polarity reveals that the enrichment of E-cadherin at edges oriented at parallel to the AP axis in WT (B) was significantly reduced in *abl* mutant embryos ($p=0.0033$) (n=3 WT, 4 *abl* mutant embryos, 53-91 edges/embryo). Values were normalized to the mean intensity of edges perpendicular (75°-90°) to the AP axis. Anterior left, dorsal up. Bar, 10 μm.

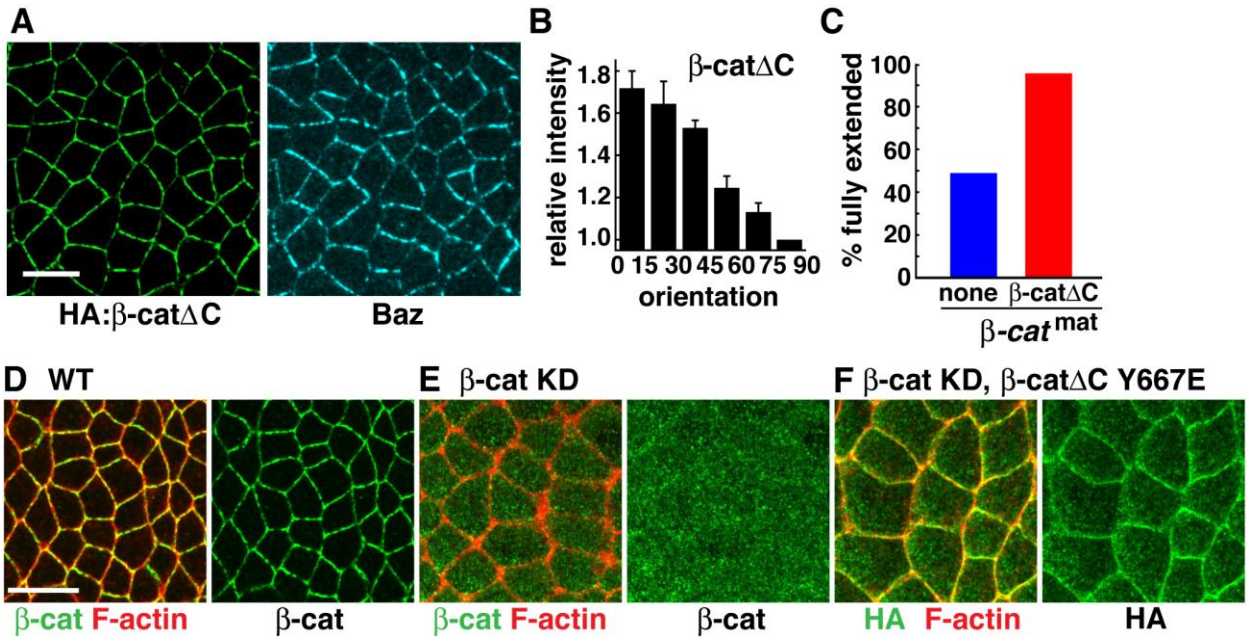

Figure S4, related to Figures 6 and 7. The β-catenin C-terminal domain is dispensable for planar polarity and axis elongation. (A,B) Planar polarized localization of HA:β-catΔC in β-catenin knockdown (β-cat KD) embryos (HA green, Baz blue). Values were normalized to the mean intensity of edges perpendicular (75–90°) to the AP axis ($p = 0.68$ vs. endogenous β-catenin) ($n = 4$ embryos, 91-114 edges/embryo). (C) HA:β-catΔC rescues axis elongation in the progeny of females bearing arm^{043A01} germline clones ($\beta\text{-}cat^{mat}$ embryos). The percentage of fully extended embryos is shown ($n = 47$ $\beta\text{-}cat^{mat}$ embryos, 90 $\beta\text{-}cat^{mat}$ embryos expressing HA:β-catΔC). Half of the embryos are predicted to receive the wild-type paternal allele, which rescues germband extension. (D,E) β-catenin expression was strongly reduced by β-cat KD (β-cat green, F-actin red). (F) HA:β-catΔC^{667E} was expressed and localized to adherens junctions in a β-cat KD embryo (HA green, F-actin red). Anterior left, dorsal up. Bars, 10 μm.

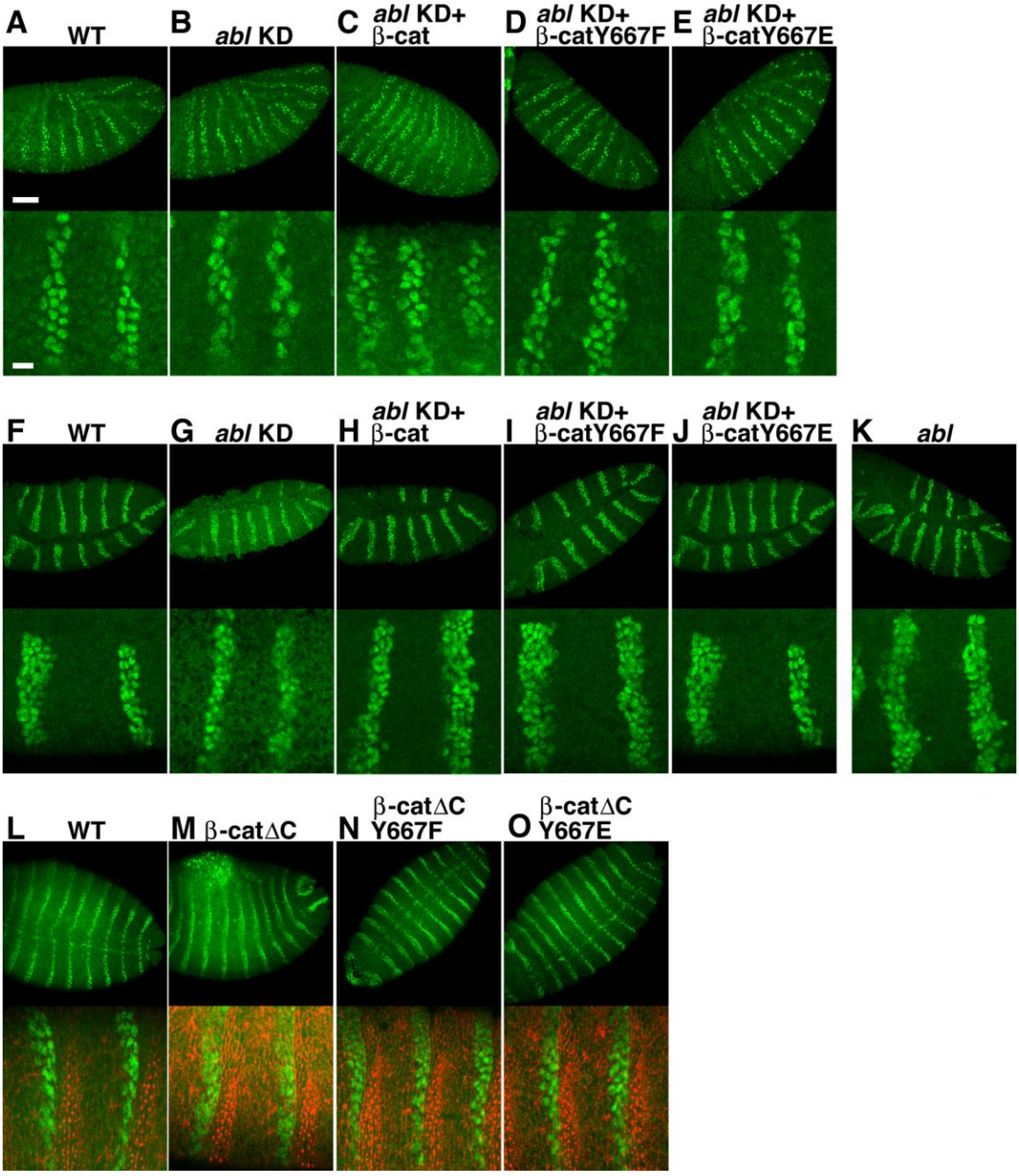

Figure S5, related to Figure 7. Wingless-dependent engrailed expression is not affected by disrupting Abl or β-catenin Y667 phosphorylation. (A-J) Embryos expressing maternal Gal4 (A,F) or maternal Gal4 driving maternal *abl* shRNA expression (*abl* KD) with or without full-length β-catenin transgenes (B-E, G-J). (K) *abl* mutant. (L-O) Embryos expressing da-Gal4 (L) or da-Gal4 driving the expression of β-catΔC transgenes (M-O). Engrailed (green), F-actin (labeling denticle precursors, red). Scale = 50 μm (top panels), 10 μm (bottom panels). Each pair of upper and lower panels represents images from a single embryo. The striped patterns of Engrailed expression and denticle formation were not altered by disrupting Abl expression or β-catenin phosphorylation on Y667.

Supplemental Experimental Procedures

Transgene construction

For pUASp-Abl:HA, the full-length *abl* cDNA was PCR amplified, cloned into pENTR/D-TOPO, and recombined into pPWH (Gateway system, Invitrogen). *β-catΔC* cDNAs were cloned into pENTR/D-TOPO and recombined into pUASp-W-attB after cloning *Venus* cDNA into the AscI site, or into pUASp-HW-attB (gifts of M. Buszczak). *β-catΔC* (aa 1-680), *β-catΔC*^{S12}, and *β-catΔC*$^{S10/150A}$ were PCR amplified from transgenic animals (Orsulic and Peifer, 1996; Hoffmans and Basler, 2007). Point mutations were made by site-directed mutagenesis (Stratagene). *β-catΔC*, *β-catΔC*^{667F}, and *β-catΔC*^{667E} tagged with Venus or HA were inserted in the attP40 site (Genetic Services). Full-length *β-catenin*, *β-catenin*^{667F}, and *β-catenin*^{667E} cDNAs were cloned into pPHW and inserted in random locations.

dsRNA- and shRNA-mediated knockdown

For dsRNA-mediated disruption of *β-catenin* expression, the DNA template to produce dsRNA was generated by PCR from genomic DNA with the following primers, each containing the T7 promoter sequence (5'-TAATACGACTCACTATAGGGAGACCAC-3') at its 5'end:
T7-forward 5'-CAAGAAGCGGCTATCCATAGAGC-3'
T7-reverse 5'-CAATCGGTATCGTACCAGGCAGC-3'
PCR product was used as the template for T7 transcription reactions (5X MEGAscript T7 Kit, Ambion). dsRNA was injected ventrally at 2 mg/ml in embryos collected 0-45 min after egg laying. Embryos were incubated in a humidified chamber at 20ºC for 2-3 hr and then mounted for time-lapse imaging.

For *abl* shRNA expression, a transgenic stock expressing *abl* shRNA from the UASp promoter in the VALIUM22 vector was obtained from the Transgenic RNAi Project at Harvard Medical School (Ni et al., 2011). This transgene was made using the following oligos:
Forward oligo 5'-
ctagcagtCACAACGATGTTAGCAACATAtagttatattcaagcataTATGTTGCTAACATCGTTGTGgcg-3'
Reverse oligo 5'-
aattcgcCACAACGATGTTAGCAACATAtatgcttgaatataactaTATGTTGCTAACATCGTTGTGactg-3'
Time-lapse imaging in *abl* shRNA-expressing embryos was performed with ubiquitously expressed E-cadherin:GFP (Oda and Tsukita, 2001) and an mCherry fusion to the myosin regulatory light chain (Sqh) (Martin et al., 2009).

Western blotting

Antibodies were mouse Arm/β-catenin (1:500), mouse PY (1:2000, Upstate 4G10), mouse GFP (1:1000, Roche), rat HA (1:1000, Roche), rat DE-cadherin (1:150, DSHB), rat α-catenin (1:500, DSHB), and rabbit Phospho-Src Family (pY416) (1:1000, Cell Signaling).

Statistical analysis

Mean values were compared using Student's t test (Glantz, 2002).

Supplemental References

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